

PATENT
10/039,956
Docket 091/009c

CLAIM AMENDMENTS

1. *(Original)* A composition comprising proliferating primate pluripotent stem (pPS) cells, which is essentially free of feeder cells.
- 2 to 10. *CANCELLED*
11. *(Original)* A method for producing differentiated cells from a donor culture of undifferentiated primate pluripotent stem (pPS) cells, comprising:
 - a) preparing a suspension of cells from the undifferentiated donor culture;
 - b) replating and culturing the suspended cells on a solid surface so that they differentiate without forming embryoid bodies; and
 - c) harvesting differentiated cells from the solid surface.
12. *(Original)* A method for producing differentiated cells from a donor culture of primate pluripotent stem (pPS) cells, comprising:
 - a) providing a culture of primate pluripotent stem (pPS) cells that is essentially free of feeder cells;
 - b) changing the medium in which the cells are cultured; and
 - c) harvesting differentiated cells after culturing for a time in the changed medium.
13. *(Previously presented)* The method of claim 11, wherein the donor culture of pPS cells is a culture essentially free of feeder cells, according to claim 1.
14. *(Original)* The method of claim 11, having at least one of the following features:
 - i) the solid surface bears a poly-cation (such as poly-lysine or poly-ornithine);
 - ii) differentiation is promoted by withdrawing serum, serum replacement, or a factor that inhibits differentiation from medium in which the cells are cultured after replating; or
 - iii) differentiation is promoted by adding a factor (such as Brain Derived Neurotrophic Factor, BDNF; or Neurtrophin-3, NT-3) that promotes differentiation in medium in which the cells are cultured after replating.
15. *(Previously presented)* A differentiated cell population produced by the method of claim 35.

PATENT
10/039,956
Docket 091/009c

16. *(Currently Amended)* A method of screening a ~~compound for cellular toxicity or modulation substance~~, comprising contacting a differentiated cell according to claim 15 ~~with the compound, with the substance, and~~ determining any phenotypic or metabolic changes in the cell that result from contact with the ~~compound~~, and ~~correlating the change with cellular toxicity or modulation substance~~.
17. *(Original)* A method for producing a polynucleotide comprising a nucleotide sequence contained in an mRNA that is expressed at a different level in committed or differentiated cells compared with undifferentiated primate pluripotent stem (pPS) cells, the method comprising:
- a) determining the level of expression of a plurality of mRNAs in committed or differentiated cells, in comparison to the level of expression of the same mRNAs in undifferentiated pPS cells;
 - b) identifying an mRNA expressed at a different level in the committed or differentiated cells, relative to the undifferentiated pPS cells; and
 - c) preparing a polynucleotide comprising a nucleotide sequence of at least 30 consecutive nucleotides contained in the identified mRNA.
- 18 to 29. **CANCELLED**
30. *(Previously Presented)* A method for producing a protein containing a sequence of a polypeptide expressed in undifferentiated or differentiated pPS cells, comprising determining amino acid sequence from a protein encoding region of an mRNA or cDNA from an expression library, and manufacturing a protein containing the determined sequence;
- wherein the expression library was obtained by providing a culture of undifferentiated pPS cells essentially free of feeder cells, optionally permitting the pPS cells to differentiate, and isolating mRNA from the undifferentiated or differentiated cells.
31. *(Previously Presented)* A method for producing an antibody specific for a polypeptide expressed in undifferentiated or differentiated pPS cells, comprising determining amino acid sequence from a protein encoding region of an mRNA or cDNA from an expression library, and immunizing an animal or contacting an immunocompetent cell or particle with a protein containing the determined sequence;
- wherein the expression library was obtained by providing a culture of undifferentiated pPS cells essentially free of feeder cells, optionally permitting the pPS cells to differentiate, and isolating mRNA from the undifferentiated or differentiated cells.
32. *(Original)* The composition of claim 1, wherein the pPS cells are human embryonic stem (hES) cells.

PATENT
10/039,956
Docket 091/009c

33 to 34. *CANCELLED*

35. *(Currently Amended)* A method for producing a population of differentiated cells, comprising:

a) obtaining a line of ~~embryonic stem cells~~ primate pluripotent stem (pPS) cells that have been established in a culture environment that is essentially free of feeder cells; and

b) optionally causing or permitting cells in the culture to differentiate into the population of differentiated cells.

36. *CANCELLED*

37. *(Currently Amended)* ~~A method of screening a substance for its effect on cultured cells~~ The method of claim 16, comprising:

a) obtaining a culture of undifferentiated pPS cells proliferating in a growth environment that is essentially free of feeder cells;

b) optionally causing or permitting the pPS cells to differentiate; then

c) combining the cells with the substance; and

d) determining any effect of the substance on the cells.

38. *(Previously Presented)* The method of claim 37, wherein the undifferentiated pPS cells are cultured on extracellular matrix components (such as Matrigel®, laminin, or collagen) in the absence of feeder cells.

39. *(Previously Presented)* The method of claim 37, wherein the cells are undifferentiated when contacted with the substance.

40. *(Previously Presented)* The method of claim 37, wherein the cells have been caused or permitted to differentiate before being contacted with the substance.

41. *(Previously Presented)* The method of claim 40, wherein the cells have been caused to differentiate by a process comprising replating them onto a surface that promotes differentiation.

42. *(Previously Presented)* The method of claim 40, wherein the cells have been caused to differentiate by adding component(s) to the medium that promote differentiation towards a particular cell lineage.

43. *(Previously Presented)* The method of claim 40, comprising causing the cells to differentiate into cells having characteristics of neuronal cells, glial cells, or neural precursors.

PATENT
10/039,956
Docket 091/009c

44. *(Previously Presented)* The method of claim 40, comprising causing the cells to differentiate into cells having characteristics of hepatocytes.
45. *(Previously Presented)* The method of claim 37, wherein the pPS cells are human embryonic stem (hES) cells.
46. *(Previously Presented)* The method of claim 37, comprising determining the effect of the substance on growth of the cells.
47. *(Previously Presented)* The method of claim 37, comprising determining whether the compound affects differentiation of the cells.
48. *(Previously Presented)* The method of claim 37, comprising determining whether the compound affects expression of a marker or receptor by the cells.
49. *(Previously Presented)* The method of claim 37, comprising determining whether the compound affects release of a marker or enzyme from the cells
50. *(Previously Presented)* The method of claim 37, comprising determining whether the compound affects DNA synthesis or repair in the cells.
51. *(Previously Presented)* The method of claim 37, comprising analyzing the cells by metaphase spread.
52. *(Previously Presented)* The method of claim 37, comprising determining whether the compound is toxic to the cells.